intronic enhancer (ASE) containing multiple FoxH1 binding sites and an Nkx2 binding site. Both binding sequences are essential and sufficient for the asymmetric enhancer activity and are evolutionarily conserved among vertebrates. Mutant mice lacking the ASE of *Pitx2* (*Pitx2*$^{\Delta ASE/\Delta ASE}$) lose left-sided Pitx2 expression and exhibit laterality defects in most of visceral organs, while the stomach and heart looping remain unaffected. Asymmetric *Pitx2* expression in some domains such as the common cardinal vein is induced by Nodal signal but is independent of the ASE. Normally, *Pitx2* is repressed in a large portion of the heart ventricle by a negative feedback mechanism at embryonic day 9.5 (E9.5), when it is still expressed in the remaining domains. This negative feedback by *Pitx2* was lost in the *Pitx2*$^{\Delta ASE/\Delta ASE}$ mice. Rescue of the early phase of asymmetric *Pitx2* expression in the left lateral plate was not sufficient for correct organogenesis, suggesting that continuous expression of *Pitx2* in the lineage of left lateral plate is required for situs-specific organogenesis.

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Spatio-temporal regulation of *Ngn2* and Hes1 by Pax3 during murine development

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Earlier studies by Koblar et al. (1999) have shown that fivefold less sensory-like neurons were generated in neural crest cultures from *Pax3* null mice as compared to wild-type littermates. The mechanism of how loss of Pax3 causes this loss of sensory neurogenesis is largely unknown. We hypothesized that Pax3 regulates the pro-neural gene *Ngn2* and neural crest stem cell maintenance gene *Hes1*. Using transient co-transfection of Pax3 expression plasmid with promoter-luciferase constructs of *Ngn2* and *Hes1*, along with chromatin immunoprecipitation and electromobility shift assays, we show that Pax3 regulates the *Ngn2* and *Hes1* promoter by directly binding to *cis*-regulatory elements. Real time quantitative RT-PCR using RNA isolated from carefully dissected rostral and caudal neural tube (separate trunk and tail portions) from individual *Pax3*$^{+/+}$ and *Pax3*$^{-/-}$ E9.5 and E11.5 embryos showed that *Ngn2* and *Hes1* are differentially expressed along the rostral–caudal axis. Differentiation of p75+ FAC sorted neural crest stem cells from *Pax3*$^{+/+}$ and *Pax3*$^{-/-}$ embryos showed early but significant reduction in neurogenesis in *Pax3*$^{-/-}$ embryos. These results show that the role of Pax3 in the regulation of neurogenesis is via regulating *Ngn2* and *Hes1* activities directly.

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Pax6 directly regulates the expression of Math5 during retinal ganglion cell differentiation

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During development of the vertebrate retina, a stratified layer of six distinct neuronal cell types is formed. Retinal ganglion cell axons project to the central nervous system and are responsible for relaying visual information to the brain. The vertebrate homologue of atonal, Math5, is required for the generation of retinal ganglion cells. Math5 mutant mice lack retinal ganglion cells and thus optic nerve projections. However, little is known about the regulatory mechanisms of Math5 expression. Analysis of Pax6 mutant mice has shown reduced expression of Math5. Here, we demonstrate that Pax6 can interact with the Math5 promoter by chromatin immunoprecipitation. Luciferase reporter constructs containing the Math5 promoter were inhibited by Pax6 transiently expressed in HEK-293 cells suggesting several regions within the Math5 promoter that are regulated by Pax6. Gel-shift analyses confirmed the ability of the Pax6 paired domain to bind to these sequences. In contrast to the genetic removal of functional Pax6 protein, our in vitro luciferase assays demonstrated that Pax6 inhibited Math5 expression. This could be due to a lack of proper interacting factors in HEK cells to activate the Math5 promoter or suggest that Pax6 is necessary but not sufficient to activate Math5 expression. A similar situation is seen in lens crystallin proteins where Pax6 acts either as a repressor or activator depending in the cellular context. Further investigation needs to be conducted to determine whether individual Pax6 binding sites are positive or negative regulators of Math5.

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Regulation of retinal ganglion cell formation: In vivo analyses of Math5 expression during mouse eye development

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The basic helix–loop–helix (bHLH) transcription factor Math5 is required for the differentiation of the first retinal neurons, retinal ganglion cells (RGCs). As this cell type relays visual input to the brain, the elucidation of Math5 regulation will contribute to understanding fundamental visual system formation. Therefore, we created transgenic mice containing different Math5 genomic DNA fragments driving a GFP